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19 ABSTRACT (Continue on reverse if necessary and identify by block number) A miniature Devanathan cell probe was modified to grow biofilms of anaerobic bacteria on palladium surfaces. Hydrogen permeation into the palladium was detected by measurement of the current passing through the cell. Permeation of the gas paralleled the growth curve of the bacteria adhering to the palladium surface. Bacteria in the liquid medium contribute minimally to hydrogen permeation. Very small populations of anaerobic bacteria growing on the palladium surface are capable of contributing significant quantities of hydrogen gas to the metal.						DISTRIBUTION STATEMENT A Approved for public release; Distribution unlimited								
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PRINCIPAL INVESTIGATOR: Ralph Mitchell

CONTRACTOR: Harvard University

CONTRACT TITLE: The Role of Microorganisms in Marine Corrosion

PERIOD OF PERFORMANCE: 12/1/87 - 11/30/88

RESEARCH OBJECTIVES:

To study the role of bacteria in marine corrosion processes. Special emphasis is being placed on the activities of hydrogen producing bacteria in embrittlement of metals.

PROGRESS:

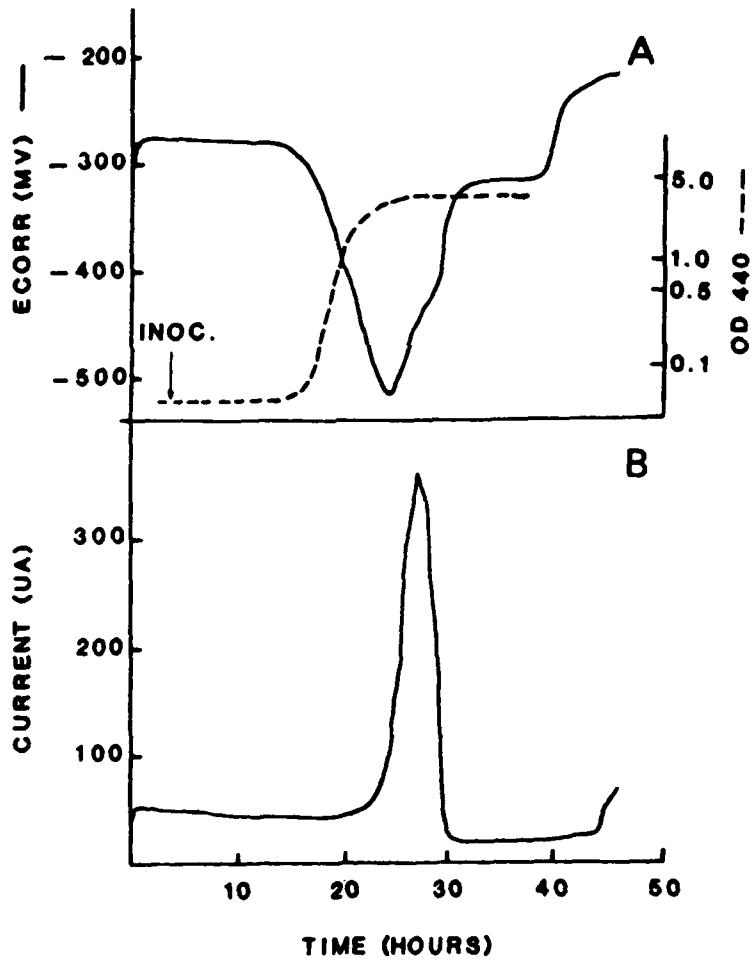
In our previous research under this contract we have grown pure cultures of anaerobic bacteria on mild steel surfaces of miniature Devanathan cells. Hydrogen gas was produced by the bacteria, and permeated the steel surface of the cell. The hydrogen permeation caused a rise in the current passing through the cell of approximately 8 to 11 ua. Substantial amounts of acetic and butyric acids were also produced by the bacterium.

During the past year we have developed a more quantitative assessment of the rate of permeation of evolved bacterial hydrogen gas through metal surfaces. Palladium was substituted for steel because of its strong absorption efficiency which is usually as high as 97%.

Figure 1 illustrates a typical growth curve of Clostridium acetobutylicum. After inoculation there is a lag phase of 8-10 hours followed by an exponential growth phase lasting approximately 3 hours, followed by stationary phase and sporulation. There is a corresponding decrease in the open circuit potential from approximately -300 millivolts to -500 millivolts (Fig. 1A). After 17 hours the hydrogen permeation current begins to increase exponentially ($r^2 = 0.94$) to 127 $\mu\text{A cm}^{-2}$ then rapidly decreases to baseline rates (Fig. 1B).

Efficiency of transport of hydrogen under these conditions was tested by electrochemically charging the input side of the membrane over the current range 18 - 176 $\mu\text{A cm}^{-2}$. Absorption

efficiency was found to be 93% over the range of currents tested ($r^2 = 99.99$). Therefore 48.6×10^{-6} mol H⁰ were produced by bacteria closely associated with the surface of the membrane. Counts of bacteria on the palladium surface were surprisingly low, averaging less than 10^6 cells cm⁻². It is probable that the absorbed hydrogen was produced by bacteria closely associated with the metal surface. This was confirmed in experiments in which 0.1um membranes were placed between the palladium and the biofilms, preventing adhesion of bacteria to the metal. Permeation currents were reduced dramatically indicating the absence of hydrogen absorption.



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Figure 1A: Typical open circuit potential characteristics of palladium during growth of Clostridium acetobutylicum. Growth measured as optical density at 440nm.

Figure 1B: Corresponding hydrogen permeation transient.

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Use of the biologically-adapted Devanathan cell enables us to study hydrogen dynamics. Nutrients, gases and bacterial numbers are being continuously monitored and related to the hydrogen permeation current. Co-cultures, tri-cultures and consortia of organisms are being studied within the system to understand the complex dynamics of mixed populations. Within a mixed microbial film the quantity of hydrogen absorbed by a metal is likely to be different from the total hydrogen produced within the film. The most important factor is the probability of hydrogen consumption by other bacteria growing on the metal surface. Hydrogen embrittlement may be determined by the outcome of competition for hydrogen between the metal and hydrogen-consuming bacteria.

OBJECTIVES FOR THE NEXT YEAR:

We plan to continue to use conventional electrochemical techniques for hydrogen permeation measurement through defined metal foils. We will continue to quantify hydrogen permeation with pure cultures of bacteria using the defined system, allowing calculations of hydrogen production on a per cell basis. Comparison will then be made with co- and mixed cultures and interspecific hydrogen transfer investigated. Effect of metabolites (e.g., sulfide, organic acids, etc.) will also be studied using the defined system. Once these reactions have been quantified with palladium, the work will extend to thin foils of metals susceptible to embrittlement, particularly high strength steels. Hydrogen permeation through these steels will be calibrated electrochemically. Permeation data will be supplemented with stress testing of steels after exposure to bacterial cultures and subsequent microstructural analysis of the metal.

PUBLICATIONS AND RESEARCH ABSTRACTS

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7. Ford, T.E., J.S. Maki and R. Mitchell, 1989, Involvement of bacterial exopolymers in biodeterioration of metals, BIODETERIORATION 7, The Biodeterioration Society, Cambridge, UK, (In Press).
8. Walch, M., T.E. Ford and R. Mitchell, 1989, Influence of hydrogen-producing bacteria on hydrogen uptake by steel, Corrosion (In Press).
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INVENTIONS:

None

TRAINING ACTIVITIES:

One graduate student and one undergraduate are working on the project.

WOMEN AND MINORITIES:

One

NON-CITIZENS:

One

AWARDS:

None